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Initiation of invasive disease in M1T1 group A streptococcus

Andrew Hollands
University of Wollongong

Initiation of Invasive Disease in M1T1 Group A Streptococcus

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Andrew Hollands

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DECLARATION

I, Andrew Hollands, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy (PhD), in the School of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Andrew Hollands

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ABSTRACT

Streptococcus pyogenes (group A streptococcus; GAS) is an important human pathogen that colonizes epithelial and mucosal surfaces. Group A streptococcal disease can be relatively minor, such as streptococcal pharyngitis, or severe and life-threatening, such as necrotizing fasciitis. There has been a resurgence of severe infection with GAS since the mid-1980s that has been paralleled be the emergence of a globally disseminated clone, M1T1. The M1T1 clone of GAS presents as the most common cause of streptococcal pharyngitis in developed countries and are also overrepresented in cases of severe infection.

Most invasive bacterial infections are caused by species that more commonly colonize the human host with minimal or no symptoms. Although phenotypic or genetic correlates underlying a bacterium's shift to enhanced virulence potential have been studied, the *in vivo* selection pressures governing such shifts are poorly understood. The globally disseminated M1T1 clone of GAS is linked with the rare but life-threatening syndromes of necrotizing fasciitis and toxic shock syndrome. Mutations in the group A streptococcal control of virulence regulatory sensor kinase (*covR/S*) operon are associated with severe invasive disease, abolishing expression of a broad spectrum cysteine protease (SpeB) and allowing the recruitment and activation of host plasminogen on the bacterial surface. This study describes how a bacteriophage-encoded group A streptococcal DNase (Sda1), which facilitates the pathogen's escape from neutrophil extracellular traps (NETs), can serve as a selective force for *covR/S* mutation. The results provide a paradigm whereby horizontal gene transfer and natural selection exerted by the innate immune system

generate hypervirulent bacterial variants with increased risk of systemic dissemination**.**

This study sought to investigate if there was a cost of fitness associated with *covR/S* mutation that counterbalances the dramatic increase in virulence. It was found that *covR/S* mutant bacteria had reduced capacity to bind fibronectin and collagen, both components of the extracellular matrix bound by streptococcal adhesins. The *covR/S* mutant strain examined in this study also showed reduced capacity to bind to epithelial cell layers as a consequence of increased capsule expression. This mutant strain displayed reduced capacity to form biofilms. An animal model of skin colonization was used to show that the *covR/S* mutant strain has a colonization defect. This reduced capacity to colonize presents an explanation as to why hypervirulent *covR/S* mutant M1T1 group A streptococci are not rapidly spread amongst the community.

The role of SpeB in the course of infection is still unclear. This study utilized a SpeB-negative M1T1 clinical isolate, 5628, with a naturally occurring mutation in the gene encoding the regulator RopB, to elucidate the role of RopB and SpeB in systemic virulence. Allelic exchange mutagenesis was used to replace the mutated *ropB* allele in 5628 with the intact allele from the well characterized isolate 5448. The inverse allelic exchange was also performed to replace the intact *ropB* in 5448 with the mutated allele from 5628. An intact *ropB* was found to be essential for SpeB expression. While the *ropB* mutation was shown to have no effect on haemolysis of RBCs, extracellular DNase activity or survival in the presence of neutrophils, strains with the mutated *ropB* allele were less virulent in murine systemic models of

infection. An isogenic SpeB knockout strain containing an intact RopB showed similarly reduced virulence. Microarray analysis found genes of the SpeB operon to be the primary target of RopB regulation. These data show that an intact RopB and efficient SpeB production are necessary for systemic infection with GAS.

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ABBREVIATIONS

°C	degrees Celsius
aa	amino acid
Ab	antibody
ANOVA	analysis of variance
APSGN	acute post-streptococcal glomerulonephritis
ARF	acute rheumatic fever
BLAST	basic local alignment search tool
bp	base pair
CCD	charge-coupled device
cDNA	complementary DNA
CFU	colony forming units
Cm	chloramphenicol
Co	collagen
CovR/S	control of virulence regulator/sensor
DNA	deoxyribonucleic acid
DTT	dithiothreitol
E. coli	*Escherichia coli*
ECM	extracellular matrix
EDTA	ethylenediaminotetraacetic acid
Erm	erythromycin
FBP	fibronectin binding protein
FCT	fibronectin-binding, collagen-binding, T-antigen
Fn	fibronectin
g	acceleration due to gravity (9.8 ms^{-2})
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GAS	group A streptococcus
GEO	Gene Expression Omnibus
h	hours
HRP	horseradish peroxidise
IgA	immunoglobulin A
IgG	immunoglobulin G
kDa	kilodaltons
LA	Luria-Bertani agar
LB	Luria-Bertani broth
LTA	lipoteichoic acid
M	molar
MBC	minimum bactericidal concentration
MF	mitogenic factor
MHC	major histocompatability complex
MIAME	minimum information about a microarray experiment
MIC	minimum inhibitory concentration
min	minutes
ml	millilitres
mM	millimolar
mm	millimetres
Mrp	M-related protein

NCBI	National Center for Biotechnology Information
NET	neutrophil extracellular trap
ng	nanogram
nm	nanometres
nt	nucleotide
OD	optical density
ORF	open reading frame
PAI	plasminogen activator inhibitor
PAM	plasminogen-binding group A streptococcal M-like protein
PBS	phosphate buffered saline
PCR	polymerase chain reaction
Prp	PAM-related protein
RBC	red blood cell
RGD	arginine-glycine-aspartic acid
RPMI	Roswell Park Memorial Institute
S. pyogenes	*Streptococcus pyogenes*
SCP	streptococcal C5a peptidase
SD	standard deviation
Sda1	streptodornase 1
SDH	streptococcal surface dehydrogenase
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEN	streptococcal surface enolase
SIC	streptococcal inhibitor of complement-mediated lysis
Ska	streptokinase
SLO	streptolysin O
SLS	streptolysin S
SmeZ	streptococcal mitogenic exotoxin Z
SOF	serum opacity factor
Spe	streptococcal pyrogenic exotoxin
SpyCEP	*Streptococcus pyogenes* cell envelope protease
SSA	streptococcal superantigen
STSS	streptococcal toxic shock syndrome
TCA	trichloroacetic acid
TCF	tissue chamber fluid
THA	Todd-Hewitt agar
THB	Todd-Hewitt broth
THY	Todd-Hewitt broth supplemented with 1% (w/v) yeast extract
TNF	tumor necrosis factor
USA	United States of America
V	volts
v/v	volume/volume
w/v	weight/volume
WT	wildtype
µl	microlitres
µm	micrometres
µM	micromolar

PUBLICATIONS

Walker, M. J., **Hollands, A.**, Sanderson-Smith, M. L., Cole, J. N., Kirk, J. K., Henningham, A., McArthur, J. D., Dinkla, K., Aziz, R. K., Kansal, R. G., Simpson, A. J., Buchanan, J. T., Chhatwal, G. S., Kotb, M. and Nizet, V. (2007). DNase Sda1 provides selection pressure for a switch to invasive group A streptococcal infection. *Nature Medicine* **13**(8): 981-5.

Hollands, A, Aziz, R. K, Kansal, R., Kotb, M., Nizet, V., Walker, M. J. (2008). A naturally occurring mutation in *ropB* suppresses SpeB expression and reduces M1T1 group A streptococcal systemic virulence. *PLoS ONE* **3**(12): e4102.

Hollands, A., Pence, M. A., Timmer, A. M., Osvath, S. R, Turnbull, L., Whitchurch, C. B., Walker, M. J., Nizet, V. The cost of evolution to a hypervirulent phenotype by M1T1 group A streptococcus is reduced capacity to colonize. *Manuscript in preparation.*

CONFERENCE PRESENTATIONS

Hollands, A., Aziz, R. K., Kansal, R., Kotb, M., Nizet, V., Walker, M. J. (2008) Mutation in *ropB* suppresses *speB* expression and reduces group A streptococcal virulence. XVII Lancefield International Symposium on Streptococci and Streptococcal Diseases. June 22-26 Porto Heli, Greece.